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IMPACT OF CONCENTRATIONS AND DIFFERENT TYPES OF SALTS IN THE GROWTH BACTERIA (RHIZOBIUM LEGUMINOSARUM IN LOAM SOILS/ THI – QAR CITY – IRAQ.)

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ABSTRACT

The study demonstrated effect of (NaCl, Na_2SO_4, $CaCl_2$, $CaSO_4$, $MgCl_2$, $MgSO_4$, KCl and K_2SO_4) on the survival of acounts of (Rhizobium Leguminosarum) presence in loam soil. Bacteria in initiation added to soil with Rate logarithmic Number amounted to (12.5) to each treatment with sterilized water in field capacity to soil sample. The loam soil was sterilized with autoclave at temperature (121 ℃) and pressure (15 bar) for half hour to three days periods to Kill all Microbes before addition the inoculation of bacteria to soil ; The inoculation of Rhizobium added to soil after sterilization with water and salts to soil for (2,4,6)weeks .The study explained that effect of all concentrations to different salts on survival rhizobia for(6) weeks incubatering with soil ,The different salts caused asignificatly reduction in log . Number of Rhizobium to all salts conparad with control treatment and with different periods of incubaton of inoculated soil with bacteria. The study explained that the log .number of bacteria were asignificant reduction to(NaCl ,$CaCl_2$, $MgCl_2$,KCl) compared with control treatment (12.5), and became (7.30 ,5.93,6.95 ,7.55) respectively and the log. Number of bectere to(Na_2SO_4,$CaSO_4$,$MgSO_4$ and K_2SO_4) became (8.41,7.10,8.52.6.78) respectively with asignifcantly reductions compared with differents salts and with control treatment. The log Number of survival bacteria with different periods (2,4,6) weeks became (7. 52 ,6,89 6.39) to chlorides salts and (8.36,7.69 and 7.18) to Sulphates salts respectively , The redultion with log Number of bacteria was signification with different periods and with control treatment. The effects Chlorides of(Na ,Ca ,Mg and K) were more than effecting of sulphates on reduction of log Number of bacteria .The study explained that The type of cation and anions were important role at decreasing or increasing log Number of bacteria in the soil. The logarithmics of Numbers of Rhizobium in inoculated soil with chlorides salts during periods (2,4,6). Weeks were (7.52 ,6.89 ,6.39) compared with Sulphates salts were (8.36 ,7.69 ,7.18) respectively . The effect of chlorides salts was more effecting than sulphates salts on survival Rhizobium in the soil.

KEYWORDS: Rhizobium Leguminosarum, Sulphates Salts, Redultion, Incubatering

INTRODUCTION

The nitrogen from an essential nutrients needed by plant (Hofman and VanCleamput,2005.) Where it enters in many biological processes and is as an essential Component in building protein (Ahmed et al ,2009) Some Soil needs to Nitrogen's fertilizer because some Nitrogen losses by leaching ,immobilization ,erosion ,Volatilization as (NH_3) and emission gases by denitrification in anerobic condition (Nielson,2006, Bundic et al 2009). Some of bacteria in soils fixes nitrogen from atmospher and very active to plant (Chiu et al,2001), The biological Nitrogen fixation is one of the most processes for ecosystem to access available Nitrogen for all living microorganisms ,Although (N_2) Consists (79%) of atmosphere ,but the triple bond between two (N) atoms is very stable and only a few group of prokaryotes can fix (N_2) to

ammonia by enzyme nitrogenase (Alexander,1982). The successful initiation of nodulation and Nitrogen Fixation by Rhizobium has two prerequisite

Infection of root hairs attachment of rhizobia to root**.**

Colonization of root hairs and Formation nodules . but stress factor such soil salinity may have an adverse effect on these two processes and limit nitrogen fixation and reducting Number of rhizobia in soils and reducting Nodule Number (Aly, etal, 2012).

The numbers of bacteria are extremely sensitive to low concentration of (NaCl) , The sensitivity is not related to rhizobial survived in soil but is probably due to the salt sensitivity of root infection sites (Singelton and Bohlool 1984) ,The salinity is an important environmental stress and posing threat to agriculture and food supply (Munns,2002,Flower,2004). it affects plant growth by osmotic effect of salts in outside solution and ion toxicity due to salt build up in transpiring leaves in second phase ,in addition to induction of nutrient deficiencies high salt stress disrupts in water potential and lons distribution head to molecular damage , growth arrest ,and even death (Zhu,2001) .Sodium toxicity under saline condition is common in graminaceous groups and results in arrange of disorders in proline Synthesis and enzyme activation (Tester and Davenport,2003).

There is bacteria (Rhizobium) in soils that have already plantes these Groups that the number of bacteria and its ability to hit the host and its ability to stabilized atmospheric nitrogen affected by the eircumstances ,including the salinity of the soil ,The Buringh(1960) pointed that the soils in Iraq were asaline and climatic was arid and Semi arid in the most area of southern of Iraq ,this conditions are suitable to varying degrees of Salinization due to climate conditions Also ,(70-80 %) of soil of central and Southern Iraq within the soils are salinity ,(Al Taie 1970), And about (50%) of lands located on the Euphrates river in Syria and Iraq affected by salt water (El Gabaly ,1970) Agriculture soils in Iraq ,accumulated amounts of salts by irrigation water year after year (Hanna,1979) As a long as this problem caused avarying degrees of salinization to the Soils at the persent time ,we must use the best practical methods and technical in order to get these soils a good yields so this study is conducting to find out the existence and survival of bacteria Rhizobium and the effect salinity by the fact the these Salinity is very important factors affecting on Southern …. Regions of Iraq and show the bacteria tolerance Salinity with different concentrations of chlorides and sulphates of(Na^{+} ,Ca^{++} ,Mg^{++} and K^{+}) in the soils.

METHODS AND MATERIALS

Location of Roots Nodules Sample

The root hairs of *vicia faba* plant brought form different locations in Thi-Qar-city (Alshaybany, collegės science , lands on side of Euphrates river and soil of electricity – power). The nodules of roots isolated from this plant growth in this locations ,cleaned sterilized ,and distroy to get bacteriods out of bodies to petri dishes and grew on yeast mannitol Agar for this bacteria to get colonies of this bacteria on the media , the colonies of cells were isolated ,purificated and transported to test tube on slant to prepare the inoculation of this bacteria and added to the soils with different types of Salts and different concentrations for different periods of incubation with Field Capacity water.

Prepare the Soil of Experiment

The soil samples were brought from the soil of college of science , Thi -Qar university to lab . cleaned , dried , grind with sieve openings (2) mm and sterilized three times for 3 days with autoclaves on (121 c̊) and (15) bar for half hour to Kill all microbes of this soil, and study chemical and physical analysis of this soil samples and explained by table

(1).

Table 1: Physical and Chemical Properties of Study Soils

Characterizes	Values	Unites
PH	7.7	-
E.C	2.29	$Ds.m^{-1}$
O.M%	0.95	%
TOC%	0.55	%
$CaCO_3$	36	%
Texture	loam	-
Sand	45	%
Clay	18	%
Silt	37	%
Ca^{++}	11.2	$Meq.L^{-1}$
Mg^{++}	6.0	$Meq.L^{-1}$
Na^{+}	5.0	$Meq.L^{-1}$
K^{+}	4.0	$Meq.L^{-1}$
Cl^{-}	14.0	$Meq.L^{-1}$
CO_3^{-}	0	$Meq.L^{-1}$
HCO_3^{-}	4.0	$Meq.L^{-1}$
$SO_4^{=}$	8.2	$Meq.L^{-1}$

Table 2: Component of Culture Media of Rhizobium

Yeast Mannitol Agar*	Amounts
Mannitol	10 gm
$K_2HPO^{-}_4$	0.8 gm
$KHPO_4^{=}$	1.0 gm
$Mg\ SO_4^{-}.\ 7H_2O$	0.4 gm
NaCl	0.1 gm
$CaCl_2$	0.1 gm
Yeast extract	1 gm
Agar	15 gm
Distilled water	1.0 liter

* yeast Mannitol broth media is without Agar.

Prepare the Initiation Inoculation of Rhizobium

The bacteria taked from slant and grew on the yeast mannitol broth media for 5 days to get The log No of bacteria amount 12.5 ,after getting The initiation log Number with 12.5 added to the soil with four treatments of concentrations of(NaCl ,Na_2SO_4, $CaCl_2$, $CaSO_4$,$MgCl_2$, $MgSO_4$, KCl and K_2SO_4).

The four concentrations of each salts were (0.25,0.5,75.1%) and with three periods (2,4,6) weeks. The loam soil used in the experiment, The component of yeast Mannitol Agar for Rhizobium explained in the table No.(2)

After addition the inoculation to the Saline soil treatment with each type of salts and incubater at 28c̊ with field water capacity for (2,4,6 weeks)

The study for survival Rhizobium in this (e) concentration of(NaCl ,Na_2SO_4 ,$CaCl_2$,$CaSO_4$,$MgCl_2$,$MgSO_4$,KCl and K_2SO_4) during three periods of incubation and recorded the log Number of bacteria (Rhizobium) to each periods and to each concentrations and found the comparations among these salts and periods and study the interactions between concentration of these salts and periods to know survival of bacteria in the soil and the tolerance of this bacteria to these

concentrations of different types salts during three periods of incubation.

The properties of physical and chemical of soil explained with table No(1):

Methods of analysis soil samplings

- Conductivity electric: measured by conductivity-meter to paste-saturation of soil according to (page et al(1982)).
- PH-soil measured by PH-meter to paste saturation according to (Black,1965).
- Carbonate and bicarbonate = measured by titration with (0.01 N H_2SO_4 Black,1965).
- Calcium and Magnesium were measured according to (Black , 1965).
- Chlorides ions : measured with titration with ($AgNO_3$) by indicator(Cr_2O_4) (Black (1965).
- Field capacity and density Bulk were measured with weightings Methods according to(Richard 1954).
- Organic Matter was measured according to walkey and Black (page *et al* 1982).
- Soil texture was measured by hydrometer according to (page *et al*1982).
- Calcium and Magnesium: Titration with EDTA and Eriechrone black (Black,1965).
- Sodium and potassium :measured by atomic Flame according to (Black ,1965.)

LABORATORY EXPERIMENTS

Sterilization of Root Nodules

The nodules were sterilized by adding them to (1/10000 $HgCl_2$ solution for(5)minutes and translated to (70%) Alcohol solution for 5minutes ,and washed by sterilized water to get from Alcohol for ten minutes ,after cleaning and sterilization of root nodule ,translated to sterilized peteri dish to Grind and distroy the cell of nodules to get bacteriods and planted them on yeast mannitol Agar enter the petri dish to get colony of bacteria ,after distroy the nodule ,was taked to do streak for purification and put on slant to prepare initiation inoculation, after preparation the inoculation of Rhizobium with yeast mannitol broth on shakers incubater for (5) day on (28ĉ) to get log Number of bacteria equal (12.5).,The inoculation added to treatment of salts to each concentrations and differents types for (2,4,6)weeks and added to soil with salts treatments .and made soil with field Capacity for yet finishing the study. The experiment analysis with factorial experiment with complete Random design (C.R.D) in spss statistical analysis and with different treatments (8×4×3×3) (AL-Rawi and Abdul –Aziz 1980).

RESULTS AND DISCUSSIONS

1- Effect of (NaCl , $CaCl_2$, $MgCl_2$ and KCl)on Rhizobium survival in the soil.

Table (3) showed the log Number of survival Rhizobia was significant reduction a($p\infty$ 0.05) after addition (NaCl ,$CaCl_2$,$MgCl_2$ and KCl) to inoculation soil and became (7.30 ,5.93 ,6.95 ,7.55) respectively , compared with log Number of Rhizobium that added to inoculation soil at the beginning of the experiment which amounts to(12.5).

The log Numbers of Rhizobium was significant reduction according to type of salt after incubation with inoculated soil because of the type of salts caused different incount of bacteria for each concentrations , The toxicity of ($CaCl_2$) more than the toxicity of Nacl in liguid and organic media (Steinborn and Roughley 1975) or caused the significatly reduction to Number of bacteria in incubater soil to different valent of element because of effecting Trivalent mor than Bi valent and more than mono valent on survival bacteria in soil (Heilman ,1975) or the salts caused high asmotic pressure and effecting on water activity to microbes in the soil Damirigi (1969) and Harris ,1981. (Roomi et al 1990).

Table 3 Effect of different Levels of Chloride Salts on Account of Bacteria during Incubation Periods

Type of salt	Conc. %	Time(Weeks)			Log No. of Bacteria	Mean
		2	4	6		
NaCl	0.25	7.4	7.20	7.0	7.20	7.30
	0.50	9.00	8.2	6.33	7.80	
	0.75	8.2	7.9	6.10	7.40	
	1.oo	8.9	6.40	5.10	6.8 0	
	∑ Xi	8.37	7.42	6.13	7.30	
$CaCl_2$	0.25	8.10	7.60	5.90	7.2 0	5.93
	0.50	7.50	6.10	4.70	6.1 0	
	0.75	7.50	6.00	4.50	6.00	
	1.oo	5.00	4.40	3.90	4.4 3	
	∑ Xi	7.02	6,02	4.75	5.39	
$MgCl_2$	0.25	8.9	8.10	7.40	8.13	6.95
	0.50	6.20	6.10	4.20	5.50	
	0.75	7.30	6.40	5.60	6.43	
	1.oo	9.60	8.30	5.40	7.76	
	∑ Xi	8.00	7.22	5.65	6.95	
KCl	0.25	9.90	9.20	8.50	9.20	7.55
	0.50	6.60	6.00	4.40	5.80	
	0.75	7.80	7.60	7.00	7.46	
	1.oo	8.60	8.40	6.30	7.76	
	∑ Xi	8.22	7.80	6.65	7.55	
Mean		7,52	6,89	6,39		

R.L.S.D(0.05)T=0.08 , C=0.08 , P=0.1, T.c=0.12, C.P=0.12, T.p=0.01, T.C.P=0.18

The salinition effected on microbes group in soil depended on types and concentration of salts(Aziz ,1986, SubbaRoa,N.S.(1999)). The table (3)showed increasing the concentration of salts in the soil led to asignificant reduction to log Number of rhizobia in soil because of increasing effect osmatic pressure on bacteria and affected on water activity to this microbes .and direct effect on Number of bacteria (AlRashidi and Fatah Alla 1983)(Hamdi et al 1975)(Neghamish 1985 ,Aziz ,1986, Flower 2004 ,Gaballeh and Gomaa 2005) All researchers were showed There were an inverse relationships between the increasing of salt concentrations and intensity microbes of population bacteria remaining in soil.

The table (3) Showed that there were significant reduction in log Number of Rhizobium in inoculated soil with increasing periods of incubation to each type and concentration of different salts, The log Number of bacteria became (7.30,5.39 ,5.95,7.55) campared with Log No of control treatment was 12.5.

The significant reduaction in log No. of bacteria was caused because of influence the survival of Rhizobium (bacteria)in soil with nutrient depletion length of the time period and put toxic waste to bacteria and its Impact on the surrounding microbial location Alexander (1982).

The figure (1) Explained ,the interaction between types of salts and concentrations caused significant reduction in log Number of Rhizobia in incubater soil The high log Number of Rhizobium became (9.20 to KCl with 0.25%).

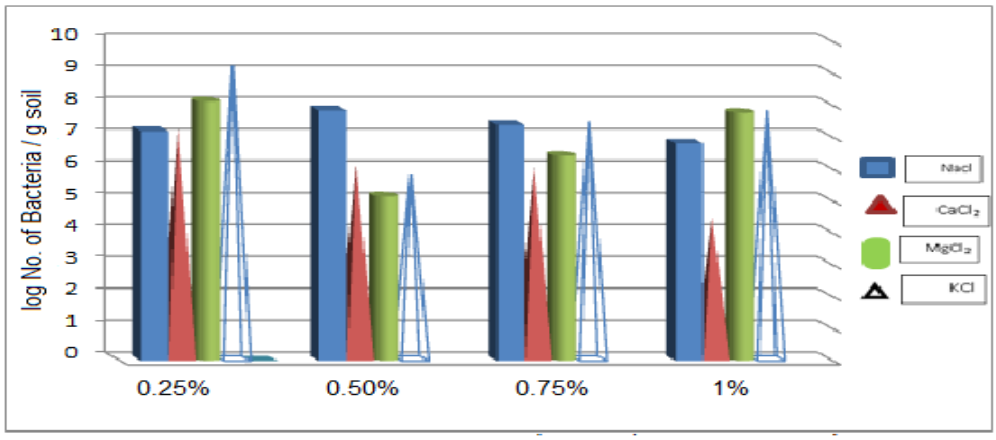

Figure 1: Effect of Interaction between Types of Chloride Salts and different Concentrations

The lowest log Number of Rhizobium in study became (4.43) to CaCl2 with concentration(1%) . The interaction between types of chlorides salts and periods figure(2) caused signifrant reduction in log Number of bacteria in inoculated soil ,The ligh log Number of Rhizobium became (8 -37) to (NaCl) with (2 weeks).

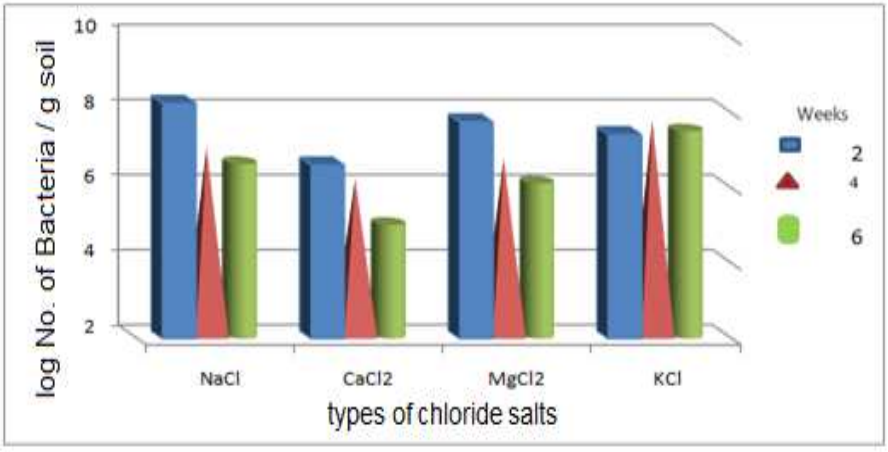

Figure 2: Effect of Interaction between Types of Chloride Salts and different Periods

The lowest log Number of Rhizobium became (4.75) to ($CaCl_2$) with (6 weeks) The interaction between concentrations of chlorides salts and periods up incubation to bacteria in the soil explained by table (3) the high log number of Rhizobium (9.90) to (KCl) with (0.25%)salt and (2) weeks the lowest log number of Rhizobium was 3.9 to 1% $CaCl_2$ with 6 week.

The interaction among typs of salts , concentration and periods ,explained by table (3) ,The higest log No of Rhizobium became (9.90) to KCl with concentration (0.25%) with period (2)weeks The lowest log Number of Rhizobium in the soil with chloride salts became (3.9)to ($CaCl_2$) with concentration (1%) and with period (6)weeks .

2- Effect of (**Na_2SO_4 , $CaSO_4$,$MgSO_4$ and K_2SO_4**) on survival Rhizobium in inoculated soil. Table (4) Showed that all types of sulphates salts (caused decreasing)with log . Number of bacteria and became (8.41,7.10,8.52 and 6.78) to (Na_2SO_4, $CaSO_4$,$MgSO_4$,K_2SO_4) respectively

Table 4: Effect of different Levels of Chloride Salts on Account of Bacteria during Incubation Periods

Type of Salts	Conc. %	Time (Weeks)			Log No. of Bacteria	Mean
		2	4	6		
Na_2SO_4	0.25	12.30	12.30	11.50	12.03	8.41
	0.50	8.00	5.40	4.90	6.10	
	0.75	6.00	6.60	10.10	7.56	
	1.oo	7.70	6.00	10	7.93	
	∑ Xi	8.50	7.60	9.12	8.40	
$CaSO_4$	0.25	9.20	7.70	7.00	7.96	7.10
	0.50	8.30	6.10	6.13	6.18	
	0.75	7.00	7.20	7.70	7.30	
	1.oo	7.60	6.10	7.20	6.96	
	∑ Xi	8.02	6.77	7.00	7.10	
$MgSO_4$	0.25	13.10	11.30	11.60	12.00	8.52
	0.50	7.80	7.60	6.20	7.20	
	0.75	5.50	7.00	10.10	7.53	
	1.oo	6.40	8.70	7.00	7.36	
	∑ Xi	8.20	8.65	8.72	8.52	
K_2SO_4	0.25	6.2	11.6	7.30	8.36	6.78
	0.50	4.40	6.40	8.60	6.46	
	0.75	2.90	6.40	8.60	5.96	
	1.oo	4.90	5.10	9.10	6.36	
	∑ Xi	4.60	7.37	8.40	6.78	
Mean		8,36	7,69	7,18		

R.L.S.D(0.05) T=0.07 , C=0.07 , P=0.09, T.c=0.1, C.P=0.15, T.p=0.08, T.C.P=0.15

The decreasing of log Number of Rhizobium was asignificant with level probability (P∝ 0.05) to each salt compared among them and compared wite control treatment which was (12.5). The ($CaSO_4$) and (K_2SO_4) were more effect on survival of bacteria in soil than the ($Na_2 SO_4$) and($MgSO_4$) The different of salt in effecting on survival of bacteria in inoculted soil because of osmotic pressure or water Activity or different with cations according to valents or effecting the salts on properties of soil like (PH), or degree of solvent the salts in the soil.

Increasing concentration of sulphate salt in the soil caused asignificant Reduction (P∝0.05) of log Number of Rhizobium in inoculted soil between them and compared with The lowest concentration ,Increasing concentrations sulphate salt caused a significant Reduction in log No . of bacteria to each salt with increasing the concentration because of increasing concentration of sulphate salt effecting on increasing osmotic pressure and effected on water activity to Microbes in soil The bacteria in soil affected by type of salts and concentration , This results liked with (Steinborn and Roughley 1975 ,Damrigi 1969,Singleton et al1982) . The logarathmics Number of bacteria (Rhizobium) were deceasing with periods ,The log Number of bacteria became (8.36 ,7.69 ,7.18) to periods (2,4,6)weeks respectively , the reason of reduction in log Number of bacteria had to nutrient depletion length of the time period and put toxic waste to bacteria and its impact on surrounding microbes location Alexander (1982).

Figure (3) explain the interaction between types of sulphate salts and concentrations caused asignificant reduction in log Number of Rhizobium in inoculated soil .

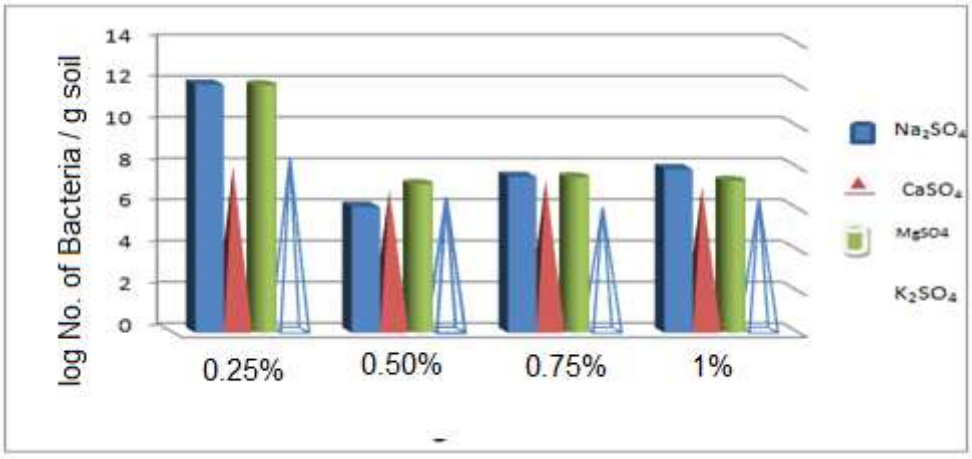

Figure 3: Effect of Interaction between types of Sulphates Salts and different Concentrations

The highest log Number of Rhizobium in inoculater soil became (12.03) and (12.0) to Na_2SO_4 and $MgSO_4$ with (0.25%) concentration . The lowest log Number of Rhizobium in inoculated soil became (5.96) to (K_2SO_4) with concentration (0.75%).

The interaction between types of Sulphates salt and periods caused asignificant reduction (P∝0.05) in log Number of bacteria in inoculated soil, The highest log Number of Rhizobium in Inoculated soil became to (9.12) to ($Na_2SO_4^-$) for (6 weeks) periods The lowest log Number of Rhizobium in the soil became (4.60)to(K_2SO_4) with(2 weeks)(figure 4).

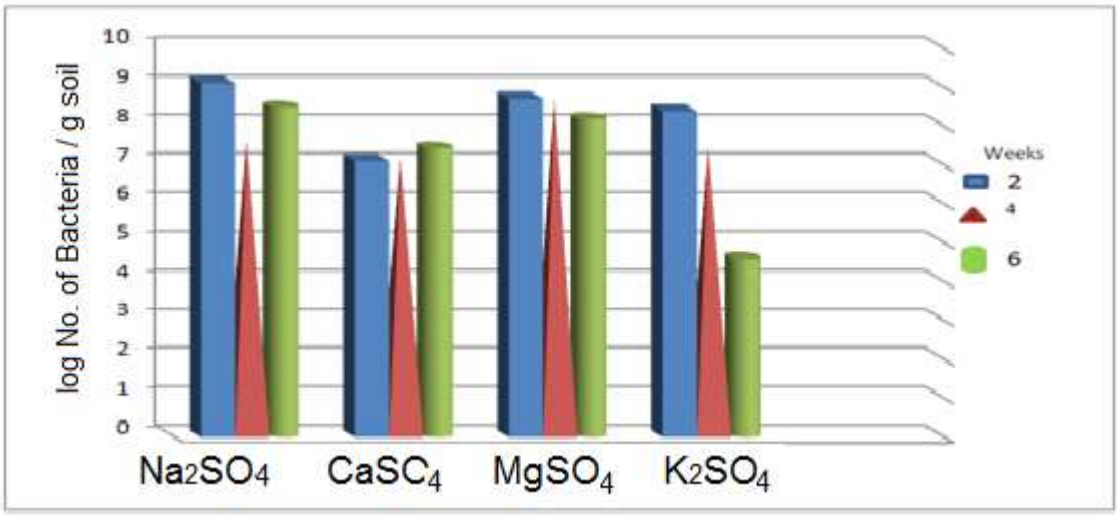

Figure 4: Effect of Interaction between Types of Sulphates Salts and different Periods

The interaction between concentrations of sulphates salts and periods of incubation to bacteria in the soil explained by (table(4)).The high log Number of Rhizobium became 13.10 to $MgSO_4$ with(2) weeks. The lowest log Number of Rhizobium in inoculated soil became(2.90) to (K_2SO_4)with(2weeks).

CONCLUSIONS

The interaction among types of salts , concentrations and periods explained by table (4). The highest log .Number of Rhizobime in inoculted soil became (13.10) with ($MgSO_4$) in concentration (0.25%) with (2weeks). And the lowest log Number of Rhizobium in soil became (2.9) to (K_2SO_4) to concentration (0.75%) for 2 weeks . The seriousness

of chlorides on microbiology bigger than sulfates because sulphates enter in food chain and enter in some amino acid like cysteine, cystine and Methonine and enter with metabolism of mineral, the sulphates effected on the properties of soil such as (PH). But chlorides have toxic to some plants we conclude from the study the salt of chlorides and suphates to (Na ,Ca ,Mg ,K) not affect completely at stopping the growth of bacteria in soil . but there is some akind of adaptation and resistance to salt of some isolates and survival in a high Level of intensity for 6 week periods ,we think that Rhizobium isolated from soline soil and have the ability to survival in more than (1%) concentration of chlorides and sulphates ,it has actually adaptability and survival in such a levels of salt , And advised to use this bacteria as abiofertilizer to soil in order to get more Nitrogen Fixation from atmosphere to fix in the soil and take by plants. The concluding effect of chlorides salts more than sulphates salts on survival bacteria but do not killing all bacteria in soil . The ($CaCl_2$) is more effecting than other salts.

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